Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.112221

CC BY

The role of fat-producing yeasts in reducing food industry waste

Dinh Tran Ngoc HUY[1,2], Trias MAHMUDIONO[3*], Nguyen Dinh Trung[4], Kadda HACHEM[5], Ahmed Remthane HUSSEIN[6], Hafsan HAFSAN[7], Gunawan WIDJAJA[8,9], Dmitry BOKOV[10,11], Aruna DHAMIJA[12], Mustafa Mohammed KADHIM[13,14,15]

Abstract

Yeasts are widely used as cellular factors in the production of bread and, more recently, various metabolic products such as vitamins, ethanol, citric acid, and lipids. Lipids synthesized by microorganisms are used in the pharmaceutical industry for technical purposes or as feed. The ability of the superior microorganism to grow on the xylose and the amount of fat production in this area were measured under optimal conditions. Xylose is one of the most abundant 5-carbon sugars in nature, and microorganisms that can grow on it are important. In this paper, the effect of different values of ammonium sulfate concentration, glucose concentration, temperature, aeration, incubation time, and pH are investigated. The results showed that with increasing ammonium sulfate concentration, glucose concentration, Incubation time, and pH, values of production of lipids, dry biomass, and percentage of lipid production by dry weight increased. Also, increasing the values of temperature and aeration has reduced the mentioned values. Finally, it can be said that the values for the studied parameters are: Concentration 1 g/L for ammonium sulfate, concentration 100 g/L for glucose, temperature 2 °C, aeration 150 rpm, incubation time 72 h, and pH equal to 6.5.

Keywords: fat-producing microorganisms; microbial oils; food industry waste; xylose.

Practical Application: In the current study, it was aimed to investigate the role of fat-producing yeasts in reducing food industry waste.

1 Introduction

Less than 5 fat-producing yeasts have been reported to have the ability to accumulate lipids of more than 25. These genera can use different carbon sources in the patrol environment and replace their fatty acids by combining their fat composition. To change (Wang et al., 2020). Yeasts have advantages over other fat-producing biological sources, and for example, their doubling time is usually less than an hour (Zhao et al., 2020). Plants are less affected by the season and climatic conditions, and their cultivation increases easily compared to algae.

Due to the diversity of microorganisms and growth conditions, fat-producing yeasts can be good sources for the production of triglycerides, surfactants, or unsaturated fatty acids (Xiong et al., 2020). This is important in producing unsaturated fatty acids for medicinal purposes or fortifying foods such as infant equations (Yun et al., 2020). These unsaturated fatty acids have significant effects on human health; for example, linolenic acid reduces the incidence of heart disease, improves cancer, obesity, diabetes, and atherosclerosis, stimulates bone formation, and lowers blood cholesterol (Wang et al., 2019; Hyun et al., 2021).

Docosahexaenoic acid is also a precursor to important signal molecules such as prostaglandins and eicosanoids. Lipid accumulation in fat-producing microorganisms occurs with cell starvation of nitrogen or other nutrients other than cranes such as phosphorus, zinc, iron, or manganese (Pini et al., 2020). The formation of lipid particles begins at the end of the logarithmic phase and continues through the dormant phase until the cranial medium begins to coagulate. In this way, lipid production in the non-crane medium occurs in a two-step

Received 23 Oct., 2021
Accepted 27 Dec., 2021
[1] *Banking University of Ho Chi Minh City, Ho Chi Minh City, Vietnam*
[2] *International University of Japan, Niigata, Japan*
[3] *Department of Nutrition, Faculty of Public Health, Universitas Airlangga, Surabaya, Indonesia*
[4] *National Economics University – NEU, Hanoi Vietnam*
[5] *Laboratory of Biotoxicology, Pharmacognosy and Biological Valorization of Plants, Faculty of Sciences, University of Saida Dr. Moulay Tahar, Saida, Algeria*
[6] *Department of Anesthesia, College of Health and Medical Technololgy, Al-Ayen University, Thi-Qar, Iraq*
[7] *Biology Department, Faculty of Science and Technology, Universitas Islam Negeri Alauddin Makassar, Gowa, Indonesia*
[8] *Faculty of Public Health, Universitas Indonesia, Depok, Indonesia*
[9] *Faculty of Law, Universitas Krisnadwipayana, Jatiwaringin, Indonesia*
[10] *Institute of Pharmacy, Sechenov First Moscow State Medical University, Moscow, Russian Federation*
[11] *Laboratory of Food Chemistry, Federal Research Center of Nutrition, Biotechnology and Food Safety, Moscow, Russian Federation*
[12] *Department of Management, GLA University, Mathura, India*
[13] *College of Technical Engineering, The Islamic University, Najaf, Iraq*
[14] *Department of Pharmacy, Osol Aldeen University College, Baghdad, Iraq*
[15] *Department of Dentistry, Kut University College, Kut, Wasit, Iraq*
*Corresponding author: sergeyv.kuznetsov@yahoo.com

process. In the first stage, the cells increase and end with the consumption of food and its. In the second phase, an excess amount of ions is converted to intracellular lipid stores. In the absence of nitrogen, fat-producing and non-fat-producing strains continue to absorb the crane, but only fat-producing organisms metabolize it and increase the cellular ATP/AMP ratio. They give existing cells, or the growth of lipid particles becomes larger (Pérez-Ortiz et al., 2019).

In non-productive yeasts, the excess crane remains unused or converted to a polysaccharide, while in fat-producing species, it converts to lipid and accumulates as tri acyl glycerol (TAG) in intracellular lipid bodies (Zhou et al., 2019). Phosphorus restriction can also play a role in the production of more lipids. When nitrogen is low in the environment, nicotine activity decreases. The metabolic pathway is altered, protein synthesis is stopped, and lipid accumulation is activated. Europa lipolytica differs from cherry-producing yeasts in biological and biotechnological applications. This microorganism is known as a model in the study of physiology, genetics, dimorphism, gene manipulation, gene expression, and lipid accumulation. This yeast differs in its ability to grow on different types of lipid-rich substrates such as dairy and meat products (Leone et al., 2019). This ability has led to the use of this yeast in the industrial production of SCO (cell oil single) or in the production of dietary supplements rich in fatty acids. Europa is a model organism for studying lipid consumption within adipocytes because not only does it have the ability to accumulate lipids in lipid particles, but the structure of this yeast cell is similar to that of eukaryotic adipocytes (Huang et al., 2021).

This yeast tends to break down its lipid accumulation. Even when fatty substrates are rich in stearic acid in the environment, it still tends to break down their lipid accumulation. Microorganisms that dazzle more than 20% of their biomass as lipids are identified as fat-producing strains, although most microorganisms accumulate a small percentage of their canvas as lipids under very appropriate conditions. Fatty residual microorganisms have the potential to be a happy substitute for vegetable oils because microbial lipids are usually in the form of triacylglycerol. Due to the abundance of lignocellulosic compounds in nature that contain carbohydrates, a good way to use natural carbohydrates as an undesirable source is to use micronutrients with a carbohydrate structure (Wu et al., 2020).

Biodiesel is one of the biofuels that has received a lot of attention in recent years due to its renewability, degradability, and less environmental pollution. Resources currently used to produce biodiesel include oilseeds, animal fats, and edible oil residues that do not meet the needs of human societies (Andrade et al., 2012). The cost of biodiesel production, most of which is related to raw materials, is one of the challenges that has limited the production of this product. Finding a solution to reduce the price of this product is being studied by many researchers (Dai et al., 2007). Microbial oils have the potential to be converted to biodiesel, and due to the advantages such as short production cycle time and ineffectiveness of seasons and climatic conditions, and also the ease of industrial production has been considered (Silva et al., 2011). Among the microorganisms, yeasts are an unsuitable suitable candidate for industrial production of biodiesel due to their advantages, such as growth rate and lipid content (He et al., 2010). Microorganisms that have a lipid accumulation capacity of more than 20% of their dry cell weight are called fat-producing (Li et al., 2007). The use of methods that can quickly and easily study the production conditions and growth of microorganisms in culture media is essential to control the production of biotechnology products (Urbina-Suarez et al., 2021).

Thus, using microbial oils, many valuable materials can be obtained in a short time and at an appropriate price. For example, the production of cocoa butter has recently been hampered by the decline of the cocoa plant, which has led to an increase in its price. However, fat-producing microorganisms have significant potential in producing oil as a substitute for cocoa butter. The lipid composition of cocoa butter is very interesting because it contains equal amounts of palmitic acid and oleic acid. This compound is rarely found in nature, but there are yeasts that can produce two traces similar to cocoa butter. Fat-producing yeasts have benefits for producing lipids over other microbial sources. For example, their doubling time is usually less than an hour, they are less affected by seasonal and climatic conditions than plants, and their cultivation increases easily compared to algae. Another advantage is that they can use different sources of fats in the culture medium. So they can change their fat composition by replacing the fatty acids in the environment. Due to the diversity of microorganisms and growth conditions, fat-producing yeasts can be a good source for the production of wind triglycerides, surfactants, and unsaturated fatty acids. It is used in the production of certain unsaturated fatty acids for medicinal purposes or to enrich foods such as infant formula.

High levels of 16 and 18 carbon unsaturated fatty acids are of great nutritional importance. These fatty acids include linolenic acid, alpha-linolenic acid, gamma-linoleic acid, oleic acid, camouflage of being acid, palmitic acid, meristic acid, arachidonic acid, eicosapentaenoic acid, docosapentaenoic acid, and deoxycholic acid. Delta 12 and delta 15 desaturase enzymes with the ability to produce linoleic acid and gamma-linolenic acid are not present in humans and animals. That is why these two fatty acids are among the essential fatty acids of some humans and animals. Inadequate nutrition in this area can affect the development of the visual system and even.

Microbial oils can be produced on a very large scale, more than 220 cubic meters. These oils are inherently safe and have passed all toxicity tests. It is accepted by all the people who use it, and no reactions against it have been reported in any way. There is no particular problem in oil extraction, and the oil can be refined. These oils are particularly resistant to oxidation, which is probably due to their natural internal antioxidants, as microbial oils with long-chain unsaturated fatty acids are used as food. Their safety in infant feeding has been investigated (Odai et al., 2019).

This oil must be obtained from non-pathogenic and non-contaminated microorganisms. Studies have shown that the use of microbial oil is as safe as the use of sunflower oil. The use of animals such as fish as a source of unsaturated fatty acids has limitations such as low and complex fatty acid content, the presence of viruses and prions in it, concerns about their

depletion, and concerns about accumulation. Contaminants such as heavy metals from marine environments in fish water follow. These cases, along with the availability of inexpensive substrates obtained from agricultural wastes, have led to more attention being paid to microbial oil or single-call oil. This is in a situation where many agricultural wastes are burned and lead to environmental pollution, and these roads signal bright horizons in the not too distant future.

2 Material and methods

This section describes how to prepare the laboratory environment and the values of the desired parameters.

2.1 Inoculum preparation

For this purpose, yeast strains are first cultured on YPD for two days. It is then transferred to a 100 mL Erlenmeyer flask containing 20 mL of pre-production medium. This environment has 15 g/L glucose and 0.5 g/L yeast extract with pH 5, placed at 30 °C and 150 rpm for 48 hours (Pan et al., 2009).

2.2 Preparation of production environment

5 mL of inoculum is combined with 45 mL of the production medium in 250 mL Erlenmeyer medium; this medium contains 50 g/L of glucose and 1 g/L of yeast extract with pH 6, for 96 hours at 30 °C and 180 rpm.

2.3 Determination of lipid and ion cell biomass

In order to determine the amount of lipids, extracellular lipids are extracted by Bligh and Dyer method. For this purpose, 50 mL of the production medium sample is centrifuged at 5000 rpm for 15 minutes. Then wash the obtained biomass twice with distilled water. Then add 10 mL of HCL in 4 mL to the desired sample and put it at 60 °C for 2 hours. Then add 20 mL of methanol-chloroform (1:1) to the acid hydrolyzed mass and allow to stand for 2 to 3 hours. Then we separate the upper and lower organic phases with aqueous centrifuges, separate the lower organic phase with the help of a pasteurizer pipette and separate the organic phase with a vacuum. The dry weight obtained shows the amount of lipid produced. In order to determine the dry cell biomass, 5 mL of the production medium at 5000 rpm for 20 minutes, after washing with water for two times, at 60 °C, until it reaches a constant weight and dries, was centrifuged.

2.4 Different values of the studied parameters

The effect of ammonium sulfate at concentrations of 0.5, 1, and 1.5 g/L on lipid production was measured. Effect of carbon source concentration (glucose) glucose concentration was changed to 50, 75, and 100 g/L, and the amount of lipid production in each concentration was investigated. Temperatures of 20 and 30 °C were also measured. Aeration was evaluated at 150 and 200 rpm. The incubation time was measured at 24, 48, 72, and 96 hours. PHs of 5, 5.5, 6, and 6.5 were measured to evaluate lipid content.

3 Results and discussion

In this section, the effect of different values of ammonium sulfate concentration, glucose concentration, temperature, aeration, incubation time, and pH on values of production of lipids, dry biomass, and percentage of lipid production by dry weigh is investigated.

3.1 Effect of ammonium sulfate concentration

The results for different values of ammonium sulfate concentration are presented in Table 1. According to the table, the values of lipids, dry biomass, and percentage of lipid production by dry weight have increased with increasing concentration. Increasing the concentration from 0.5 to 1 g/L, the values of production of lipids, dry biomass, and percentage of lipid production by dry weigh increased by 9.95, 1, and 11.07%, respectively, and with increasing the concentration from 1 to 1.5 g/L, the mentioned values have decreased by 7.17, 10.05 and 2.16%, respectively.

3.2 Effect of glucose concentration

The results for different values of glucose concentration are presented in Table 2. According to the table, with increasing the concentration of glucose, from 50 to 75 g/L, the values of production of lipids, dry biomass, and percentage of lipid production by dry weight have increased by 25.82, 8.74, and 15.69%, respectively. Also, with increasing the concentration from 75 to 100 g/L, the values of production of lipids and percentage of lipid production by dry weight decreased by 8.21, 9.62%, respectively, and the value of dry biomass increased by 1.6%.

3.3 Effect of different temperature values

The results for temperatures 20 and 30 °C are presented in Table 3. According to the table, the values of lipids, dry biomass, and percentage of lipid production by dry weight have decreased with increasing temperature. With increasing temperature from 20 to 30 °C, the mentioned values have decreased by 23.66, 18.94, and 5.83%, respectively.

3.4 Effect of different aeration values

The results for different amounts of aeration are presented in Table 4. According to the table, with increasing the amount

Table 1. Effect of different values of ammonium sulfate concentration.

Ammonium sulfate concentration (g/L)	Production of lipids (g/L)	Dry biomass (g/L)	Percentage of lipid production by dry weight
0.5	7.74	18.72	40.93
1	8.51	18.91	45.46
1.5	7.9	17.01	46.44

of aeration, the values of production of lipids, dry biomass, and percentage of lipid production by dry weight have decreased. With increasing the amount of aeration from 150 to 200 rpm, the mentioned values have decreased by 13.85, 1.45, and 12.57%, respectively.

3.5 Effect of different incubation time values

The results for the different values of incubation time are presented in Table 5. According to the table, with increasing the value of incubation time from 24 to 72 h, the values of production of lipids, dry biomass, and percentage of lipid production by dry weigh have increased equal to 98.9, 82.48, and 14.68%, respectively. Also, with increasing incubation time from 72 to 96 h, the mentioned values have decreased by 10.21, 11.23, and 0.39%, respectively.

3.6 Effect of different pH values

The results for different pH values are presented in Table 6. According to the table, increasing the pH value has increased the values of production of lipids, dry biomass, and percentage of lipid production by dry weight. With increasing the pH value from 5 to 6.5, the mentioned values have increased equal to 10.77, 4.32, and 6.6%, respectively.

Finally, it can be said that the values for the studied parameters are: Concentration 1 g/L for ammonium sulfate,

Table 2. Effect of different values of glucose concentration.

Glucose concentration (g/L)	Production of lipids (g/L)	Dry biomass (g/L)	Percentage of lipid production by dry weight
50	6.39	16.71	38.24
75	8.04	18.17	44.24
100	7.38	18.46	39.98

Table 3. Effect of different temperature values.

Temperature (°C)	Production of lipids (g/L)	Dry biomass (g/L)	Percentage of lipid production by dry weight
20	9.13	19.64	46.49
30	6.97	15.92	43.78

Table 4. Effect of different aeration values.

Aeration (rpm)	Production of lipids (g/L)	Dry biomass (g/L)	Percentage of lipid production by dry weight
150	9.1	17.23	52.81
200	7.84	16.98	46.17

Table 5. Effect of different incubation time values.

Incubation time (h)	Production of lipids (g/L)	Dry biomass (g/L)	Percentage of lipid production by dry weight
24	4.53	9.42	48.09
48	6.62	13.22	50.08
72	9.01	17.19	55.15
96	8.09	15.26	53.01

Table 6. Effect of different pH values.

PH	Production of lipids (g/L)	Dry biomass (g/L)	Percentage of lipid production by dry weight
5	9.47	17.36	54.33
5.5	9.62	17.43	55.41
6	9.88	17.69	55.85
6.5	10.49	18.11	57.92

concentration 100 g/L for glucose, temperature 2 °C, aeration 150 rpm, incubation time 72 h, and pH equal to 6.5.

4 Conclusion

According to the results, the fat-producing yeast Cryptococcus Albidus has a high ability to produce microbial oil. This, along with the ability of this yeast to use a source of nylon and, in addition to the use of agricultural waste, makes it more important. The results showed that with increasing ammonium sulfate concentration, glucose concentration, Incubation time, and pH, values of extraction of lipids, dry biomass, and percentage of lipid production by dry weight increased. Also, increasing the values of temperature and aeration has reduced the mentioned values. Finally, the results express indicate the high potential of this yeast for use in industrial applications.

References

Andrade, R., Leal, R., Roseiro, J., Reis, A., & Silva, T. L. (2012). Monitoring Rhodosporidium toruloides NCYC 921 batch fermentations growing under carbon and nitrogen limitation by flow cytometry. *World Journal of Microbiology & Biotechnology*, 28(3), 1175-1184. http://dx.doi.org/10.1007/s11274-011-0920-2. PMid:22805838.

Dai, C.-C., Tao, J., Xie, F., Dai, Y.-J., & Zhao, M. (2007). Biodiesel generation from oleaginous yeast *Rhodotorula glutinis* with xylose assimilating capacity. *African Journal of Biotechnology*, 6(18), 2130-2134.

He, M.-X., Hu, Q., Gou, X., Liu, X., Li, Q., Pan, K., Zhu, Q., & Wu, J. (2010). Screening of oleaginous yeast with xylose assimilating capacity for lipid and bio-ethanol production. *African Journal of Biotechnology*, 9(49), 8392-8397.

Huang, L., Zhang, M., & Xiong, J. (2021). Study on the mechanism of rhynchophylline in improving obese hypertension through JAK2/STAT3 pathway. *Food Science and Technology*, 1-6. Ahead of print. http://dx.doi.org/10.1590/fst.33121.

Hyun, Y. J., Kim, J. G., Kim, M. J., Jung, S. K., & Kim, J. Y. (2021). Mineral-rich Jeju lava sea water suppresses lipid accumulation in 3T3-L1 adipocytes and ameliorates high-fat diet-induced obesity in C57BL/6 J mice. *Food Science and Biotechnology*, 30(2), 299-304. http://dx.doi.org/10.1007/s10068-020-00859-8. PMid:33732520.

Leone, A., Longo, C., Gerardi, C., & Trosko, E. (2019). Pro-apoptotic effect of grape seed extract on MCF-7 involves transient increase of gap junction intercellular communication and cx43 up-regulation: a mechanism of chemoprevention. *International Journal of Molecular Sciences*, 20(13), 3244. http://dx.doi.org/10.3390/ijms20133244. PMid:31269652.

Li, Y., Zhao, Z., & Bai, F. (2007). High-density cultivation of oleaginous yeast *Rhodosporidium toruloides* Y4 in fed-batch culture. *Enzyme and Microbial Technology*, 41(3), 312-317. http://dx.doi.org/10.1016/j.enzmictec.2007.02.008.

Odai, T., Terauchi, M., Kato, K., Hirose, A., & Miyasaka, N. (2019). Effects of grape seed proanthocyanidin extract on vascular endothelial function in participants with prehypertension: a randomized, double-blind, placebo-controlled study. *Nutrients*, 11(12), 2844. http://dx.doi.org/10.3390/nu11122844. PMid:31757033.

Pan, L.-X., Yang, D.-F., Shao, L., Li, W., Chen, G.-G., & Liang, Z.-Q. (2009). Isolation of oleaginous yeast from the soil and studies of their lipid-producing capacities. *Food Technology and Biotechnology*, 47(2), 215-220.

Pérez-Ortiz, J. M., Alguacil, L. F., Salas, E., Hermosín-Gutiérrez, I., Gómez-Alonso, S., & González-Martín, C. (2019). Antiproliferative and cytotoxic effects of grape pomace and grape seed extracts on colorectal cancer cell lines. *Food Science & Nutrition*, 7(9), 2948-2957. http://dx.doi.org/10.1002/fsn3.1150. PMid:31572588.

Pini, F., Aquilani, C., Giovannetti, L., Viti, C., & Pugliese, C. (2020). Characterization of the microbial community composition in Italian cinta senese sausages dry-fermented with natural extracts as alternatives to sodium nitrite. *Food Microbiology*, 89, 103417. http://dx.doi.org/10.1016/j.fm.2020.103417. PMid:32138987.

Silva, T. L., Feijão, D., Roseiro, J. C., & Reis, A. (2011). Monitoring Rhodotorula glutinis CCMI 145 physiological response and oil production growing on xylose and glucose using multi-parameter flow cytometry. *Bioresource Technology*, 102(3), 2998-3006. http://dx.doi.org/10.1016/j.biortech.2010.10.008. PMid:21030251.

Urbina-Suarez, N. A., Barajas-Solano, A. F., Garcia-Martinez, J. B., Lopez-Barrera, G. L., & Gonzalez-Delgado, A. D. (2021). Cultivation of *Chlorella* sp. for biodiesel production using two farming wastewaters in eastern Colombia. *Journal of Water and Land Development*, 50, 141-149.

Wang, L., Huang, W., & Zhan, J. (2019). Grape seed proanthocyanidins induce autophagy and modulate survivin in HepG2 cells and inhibit xenograft tumor growth in vivo. *Nutrients*, 11(12), 2983. http://dx.doi.org/10.3390/nu11122983. PMid:31817589.

Wang, Y., Wang, Y., Shen, W., Wang, Y., Cao, Y., Nuerbulati, N., Chen, W., Lu, G., Xiao, W., & Qi, R. (2020). Grape seed polyphenols ameliorated dextran sulfate sodium-induced colitis via suppression of inflammation and apoptosis. *Pharmacology*, 105(1-2), 9-18. http://dx.doi.org/10.1159/000501897. PMid:31743904.

Wu, L., Weng, M., Zheng, H., Lai, P., Tang, B., Chen, J., & Li, Y. (2020). Hypoglycemic effect of okra aqueous extract on streptozotocin-induced diabetic rats. *Food Science and Technology*, 40(4), 972-978. http://dx.doi.org/10.1590/fst.28619.

Xiong, Y., Chen, M., Warner, R. D., & Fang, Z. (2020). Incorporating nisin and grape seed extract in chitosan-gelatine edible coating and its effect on cold storage of fresh pork. *Food Control*, 110, 107018. http://dx.doi.org/10.1016/j.foodcont.2019.107018.

Yun, S., Chu, D., He, X., Zhang, W., & Feng, C. (2020). Protective effects of grape seed proanthocyanidins against iron overload-induced renal oxidative damage in rats. *Journal of Trace Elements in Medicine and Biology*, 57, 126407. http://dx.doi.org/10.1016/j.jtemb.2019.126407. PMid:31570250.

Zhao, X., Chen, L., Wu, J., He, Y., & Yang, H. S. (2020). Elucidating antimicrobial mechanism of nisin and grape seed extract against Listeria monocytogenes in broth and on shrimp through NMR-based metabolomics approach. *International Journal of Food Microbiology*, 319, 108494. http://dx.doi.org/10.1016/j.ijfoodmicro.2019.108494. PMid:31918346.

Zhou, F., Yin, M., Liu, Y., Han, X., Guo, J., Ren, C., Wang, W., Huang, W., Zhan, J., & You, Y. (2019). Grape seed flour intake decreases adiposity gain in high-fat-diet induced obese mice by activating thermogenesis. *Journal of Functional Foods*, 62, 103509. http://dx.doi.org/10.1016/j.jff.2019.103509.